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Review Article

Molecular mechanisms of stem cell therapy in alcoholic liver disease

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ABSTRACT

Alcoholic liver disease affects a great number of people worldwide. With limited therapeutic options, stem cell therapy offers significant potential for these patients. To date, a limited number of clinical trials have produced transient clinical responses to cell therapy in patients with alcoholic liver disease. Stem cell therapy to reorganize the postnatal liver is an important theme and mission for patients with chronic liver disorders including alcoholic liver injury. We therefore should redevelop the evidence of cell-based liver regeneration therapy, focusing on targets (disease, patient's status and hepatic function), materials (cells, cytokines and genes), and methodology (stem cell types and their derived microparticles, transplantation route, implantation technology and tissue engineering). In this review, we summarize the recent findings regarding the experimental and clinical use of mesenchymal and liver stem cells, focusing mainly on the treatment of alcoholic liver disorders and their relevance in the field of regenerative medicine, and advances on the role of microvesicles and exosomes in this process. We discuss new advances in stem cell therapy from liver regeneration to liver re-organization, which is involved in the recent progress of on-going clinical trials, basic research in stem cell therapy and liver regeneration, and updated exosomes/microvesicles recovery/repairing technology.

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1. Introduction

Chronic alcohol consumption is a major cause of liver disease. The term alcoholic liver disease (ALD) refers to a spectrum of mild to severe disorders including steatosis, steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Ethanol impairs hepatic regeneration by acting directly as a hepatotoxin and dysregulating numerous important hepatic functions [1]. Some major areas of recent research have emphasized the pathogenesis of ALD through oxidative stress, glutathione depletion, abnormal methionine metabolism, malnutrition, the leakage of intestinal endotoxins, lipopolysaccharide (LPS) signaling, innate immunity, and transcription factors [2–9].

In the United States, the most recent published Centers for Disease Control and Prevention (CDC) data from 2010 ranks liver disease as the 12th leading cause of death (total 31,802) with alcoholic cirrhosis accounting for 50% of all cases [10]. Liver transplant is the only definitive treatment for patients with decompensated disease. However, the scarcity of donor organs and complications associated with immunosuppression and rejection limits its availability and clinical utility. Stem cell therapy has emerged as a promising investigational treatment modality of ALD. This article reviews the dysregulated molecular mechanisms of liver regeneration in ALD, and critically examines the clinical trials of stem cell therapy in patients with ALD to date.

2. Liver regeneration in ALD

The role of hepatocytes, human hepatic stem cells (HpSCs) and bone marrow stem cells have been extensively studied in liver repair. Three mechanisms have been identified behind the remarkable regenerative capacity of the liver. There is undoubtedly overlap between the temporal contributions of the cellular responses, and their functions are intrinsically connected. However, to engage the complex biopathology of ALD, the separation of these interrelated mechanisms represents a convenient and hopefully useful reductive intellectual framework. The emerging role of paracrine factors

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promoting proliferation of surviving intrinsic epithelial cells is discussed separately.

2.1. Hepatocyte response and its dysfunction in ALD

As the first line of defense, liver damage from any etiology induces the proliferation of mature hepatocytes with minimal involvement from liver stem cells [11–13]. This has been modeled primarily by 70% partial hepatectomy in mice and rats. Rather than true regeneration, this process is a compensatory hyperplastic response with some loss of the liver's gross anatomy and architecture. Normally, hepatocytes undergo 2–3 divisions per year, but in a hepatectomy mouse model, 70–90% of hepatocytes may begin DNA synthesis and proliferation in the first 48 h [14]. It has long been established that ethanol exposure blocks this first line cellular response. Both acute and chronic ethanol exposure have been shown to inhibit DNA synthesis in the regenerating liver and cultured hepatocytes [15,16].

Impaired hepatocyte proliferation is the consequence of oxidative damage from the reactive oxygen species produced in alcohol metabolism [17]. As cellular antioxidants decrease with an increase in the cellular NADH/NAD^+ ratio, hepatocytes exhibit decreased replication and viability [18]. Chronic alcohol intake blocks the hepatocyte regenerative response to multiple cytokine pathways. Normal quiescent hepatocytes express growth receptors for hepatocyte growth factor (HGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and stem cell factor receptor (c-Kit). Of these ligands, only HGF receptor and ligands of the EGF receptor (EGFR), namely TGFα, amphiregulin, and heparin-binding EGF-like growth factor (HBEGF) have been defined as direct mitogens producing a clonal expansion of hepatocytes in serum-free media [19].

Ethanol exposure has been shown to inhibit receptors for both HGF and EGF. HGF is a mitogenic peptide, known for its autocrine and paracrine functions. This peptide is secreted by perisinusoidal mesenchymal cells and acts on the tyrosine kinase receptor encoded by cMet [20]. HGF acts primarily upon epithelial cells and endothelial cells in cellular growth, motility, and it may also have a role in the recruitment of hematopoietic progenitor cells – which will be discussed later. Following ethanol exposure and subsequent treatment with HGF, hepatocytes exhibit impaired DNA synthesis and calcium mobilization compared to controls [21].

Furthermore, hepatocytes treated with ethanol exhibit a decreased response to EGF. These cells exhibit both reduced binding capacity of epidermal growth factor related to decreased expression of its receptor, and impaired receptor function in subsequent autophosphorylation, and receptor endocytosis [22,23]. The defective EFGR function inhibits G_1–S phase entry with delayed transcriptional activation and reduced protein expression of cyclin-dependent kinases [24]. In conjunction with impaired HGFR function, this likely explains the ethanol-exposed hepatocyte phenotype of cell senescence or cell cycle arrest.

Other substances including TNF-α, IL-6, serotonin and norepinephrine have been identified as indirect mitogens of hepatocytes. These have been extensively reviewed but have not been shown to directly initiate cell replication [25]. For example, ethanol intake disrupts proregenerative signals of TNF-α/NF-κB pathway and IL-6 with downstream inhibition of the leucine zipper transcription factor, c-jun and cyclin dependent kinases. These effects are mediated through either EGFR or HGFR [26,27]. In general, the inhibition of hepatocyte regeneration in ALD forces the activation of secondary stem cell mechanisms, which have been associated with the pathologic progression of ALD.

2.2. Hepatic stem cell response

Following the dysfunction of hepatocyte hyperplasia, a second-line response to cellular injury is observed. Severe injury from ALD causes increased proliferation of hepatic stem cells (HpSCs) [28]. These multipotent cells are derived from the embryonic cells of the ductal plate. The cells are identified from their expression of progenitor markers: claudin 3, neural cell adhesion molecule (NCAM), or hematopoietic, endothelial, or mesenchymal cell markers, which are not found on hepatoblasts [12]. These cells are located in stem cell niches in the Canals of Hering, anatomically juxtaposed between the intralobular canalicular system of hepatocytes and the biliary tree [29]. Extrahepatically, the HpSCs are located along the common pancreatobiliary ducts and in glands throughout the peribiliary system.

Hepatic stems cells respond to severe injury through a process termed activation beginning with rapid proliferation and lineage-restriction into hepatoblasts (historically known as "oval cells"), a transit-amplifying stem cell line analogous to fetal hepatoblasts [30–32]. These bipotential cells have been identified by higher levels of albumin, AFP, P450 A7, and intercellular adhesion molecule 1 (ICAM-1). They give rise to either hepatocytes or cholangiocytes. The proliferation and differentiation of these cells has been previously identified as the so-called "ductular reaction" [29,33].

The accumulation of hepatoblasts has been reported in both human and animal livers in response to severe liver injury [13,34,35]. The "ductular reaction" pattern of hepatic recovery is now thought to be stereotypical of a severe liver injury in general – rather than solely ethanol related. In acute alcoholic hepatitis, the expression of cytokeratin-7, CD-133 and epithelial cell adhesion molecule correlates with poor prognosis, but their exact role in disease progression remains unclear [36]. In patients with ALD, hepatoblast proliferation relates directly to the degree of liver damage and the extent of fibrosis [13]. This activity is mediated through the Wnt/β-catenin and sonic hedgehog pathways, which are also known pathways in stem cell differentiation and oncogenesis [28,37–39].

The degree of cytokine activation and dysregulation of hepatic stem cells has been a major focus of research in cirrhosis and alcoholic liver diseases, as related to stem cell activation and the process of hepatic fibrogenesis [40]. Stems cells are the primary source of fibrillar collagens and other extracellular matrix proteins whose pathologic expression characterizes liver fibrosis. While the exact contribution of stems cells to fibrosis remains unclear, studies on stem cell therapies may have a role in limiting the excess fibrin and collagen production [41].

2.3. Bone marrow stem cell response

More than a decade ago, a series of landmark studies demonstrated the participation of bone marrow stem cells in hepatic regeneration [42–44]. In 2000, Theise et al. studied cadaveric livers from 2 female patients, who had received male bone marrow transplants [45]. Fluorescence *in situ* hybridization (FISH) was used to identify y-chromosomes in 4% and 43% of hepatocytes. These cells were noted to be positive for hematopoietic stem cell markers: CK8, CK18, and CK19. The elevated percentage of donor-derived hepatocytes was found in a patient with chronic hepatitis C, which is consistent with subsequent observations that stem cells are not significantly involved in regeneration outside of chronic or severe hepatic injury.

Since this discovery, a great deal of research has focused on the regenerative potential of bone marrow stem cell therapy. Bone marrow stems cells are capable of differentiating into hematopoietic or mesenchymal stem cell lineages. These cell lines and multipotent adult progenitor cells have demonstrated a capacity